Maternal energy allocation to offspring increases with environmental quality in house mice

Schubert, Kristin A.; de Vries, Gonda; Vaanholt, Lobke M.; Meijer, Harro A.J.; Daan, Serge; Verhulst, Simon

Published in:
American Naturalist

DOI:
10.1086/598495

Maternal Energy Allocation to Offspring Increases with Environmental Quality in House Mice

Kristin A. Schubert,[1,*] Gonda de Vries,[1] Lobke M. Vaanholt,[1,2] Harro A. J. Meijer,[3] Serge Daan,[1] and Simon Verhulst[1]

1. Behavioral Biology Group, Center for Behavior and Neurosciences, University of Groningen, Groningen, The Netherlands;
2. Energetics Group, Institute for Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, United Kingdom;
3. Centre for Isotope Research, University of Groningen, Groningen, The Netherlands

Submitted October 22, 2008; Accepted January 13, 2009; Electronically published April 17, 2009

ABSTRACT: Breeding success generally increases with environmental productivity, but little is known about underlying mechanisms, and such relationships are not quantitatively understood. We studied female mice reproducing across an experimental environmental-quality gradient defined by the amount of wheel running required to obtain a food reward. Measuring energy metabolism with doubly labeled water, we quantified how mice made two key decisions: how much food to earn and how to allocate the energy earned between self and offspring. As environmental quality declined, female foraging effort increased, but not sufficiently to compensate for the increase in foraging costs. In absolute terms, energy allocated to both self and offspring was lower in a poor-quality environment. Moreover, the proportion of gained energy that was allocated to offspring declined with decreasing environmental quality. Environmental effects on reproductive output (total litter mass produced) could be fully explained by energy allocated to milk. Thus, the efficiency with which offspring converted milk energy to tissue growth was independent of environmental quality. To the best of our knowledge, this is the first study to provide a quantitative explanation, via maternal energy allocation, of the link between foraging costs and reproductive output.

Keywords: life history, allocation trade-offs, lactation energetics, cost of reproduction, foraging costs, parental effort.

Introduction

It is widely acknowledged that the abundance of food has notable effects on reproductive output in many animal species (birds: Martin 1987; mammals: Boutin 1990). Less well established are the mechanisms by which these effects arise and the consequences of food abundance for overall food consumption of individuals and populations. Reproduction requires a large energetic commitment (Gittleman and Thompson 1988; Williams and Vézina 2001; Speakman and Król 2005), and a fundamental prediction from life-history theory is that energy allocation to competing processes—such as foraging activity, maintenance of body tissues, and offspring provisioning—constitutes an optimal solution to the problem of resource limitation (Roff 1992; Stearns 1992). Understanding the factors that shape reproductive investment requires an approach integrating environmental conditions with physiological aspects of reproduction (Ricklefs and Wikelski 2002; Speakman 2008). Such an understanding is required to make predictions regarding the consequences of environmental quality for reproductive output (e.g., using individual-based models), which may be important in conservation measures, the study of population dynamics, and modeling the evolution of reproductive strategies.

Theoretical studies have emphasized the importance of linking environmental quality with energy allocation to reproduction (Boggs 1992; de Jong and van Noordwijk 1992), and experimental studies exploring this relationship by manipulating environmental quality have documented concurrent changes in reproductive output (i.e., wild mice *Peromyscus maniculatus* and *Mus musculus*: Perrigo 1987; zebra finches *Taeniopygia guttata*: Lemon 1991; Palestine sunbirds *Nectarinia osea*: Markman et al. 2002). However, these studies did not make a mechanistic link between environmental quality and energy allocation to reproduction. Thus, it is not clear exactly how, in a quantitative sense, improved foraging conditions enhanced reproductive success. This issue can be addressed by studying maternal energy allocation in situations where foraging costs per reward differ (i.e., in habitats of different quality). Mammalian breeding is an interesting model for investigating this problem, because reproductive effort is fully quantifiable on the basis of maternal energy intake and milk production (Daan et al. 1991; Sikes 1995).

We set out to understand the mechanism underlying the relationship between environmental quality and reproductive output by quantifying energy allocation of re-

* Corresponding author; e-mail: k.a.schubert@rug.nl.

Am. Nat. 2009. Vol. 173, pp. 831–840.
0003-0147/2009/17306-50823$15.00.
DOI: 10.1086/598495

producing female mice across an environmental-quality gradient. Environmental quality was defined as the foraging effort required to obtain a food reward. In figure 1, we provide a schematic overview of the array of possible solutions to two key decisions faced by lactating female mice: (1) how much to forage to obtain food (*top two rows*) and (2) how to allocate the energy they earned between self and offspring (*bottom two rows*). They can adjust their foraging effort (fig. 1, *top row*) to different degrees to obtain either lower, equal, or higher gross gains (fig. 1, *second row*). Given the energy spent on activity and the resultant gain, they are faced with the decision of whether to allocate resources with priority to themselves (fig. 1, *third row*) or to their offspring (fig. 1, *bottom row*) or to adopt some form of intermediate compromise. The foraging activity depicted at the top and the subsequent allocation decisions shown at the bottom of the figure directly influence maternal daily energy expenditure (DEE) and energy allocation to reproduction (in mammals: milk energy output [MEO]). DEE incorporates both body maintenance costs and the costs of foraging activity (*dashed lines*).

These schematic models illustrate that, within their range of behavioral responses to environmental conditions, animals can choose a continuum of metabolic strategies ranging from no compensation to overcompensation. The latter case is predicted when animals increase activity to obtain enough energy to cover the metabolic demands of that activity. Furthermore, prior studies have found that nonbreeding mammals and birds increase activity with rising foraging costs but, with one exception (Wiersma et al. 2005), to a level insufficient to maintain their energy intake (reviewed in Wiersma and Verhulst 2005; Vaanholt et al. 2007; Schubert et al. 2008). This makes it reasonable to expect that breeding animals will follow an intermediate strategy of "partial compensation" for elevated foraging costs (i.e., show behavior intermediate between the first two columns of fig. 1).

We empirically evaluated these allocation models by manipulating foraging costs per food reward (hereafter termed simply "foraging costs") in reproducing female laboratory mice from the third week of gestation through weaning. We measured the reproductive consequences of foraging-cost variation, quantifying litter size, growth, and survival. We further dissected maternal energy allocation to foraging activity plus somatic maintenance, on the one hand, and energy provided to offspring as milk, on the other hand. This was done using simultaneous measurements of total energy intake (on the basis of food consumption) and daily energy expenditure (using the doubly labeled water method; Speakman 1997); the difference between the two provided an estimate of the energy allocated to milk (Król and Speakman 2003). We further determined

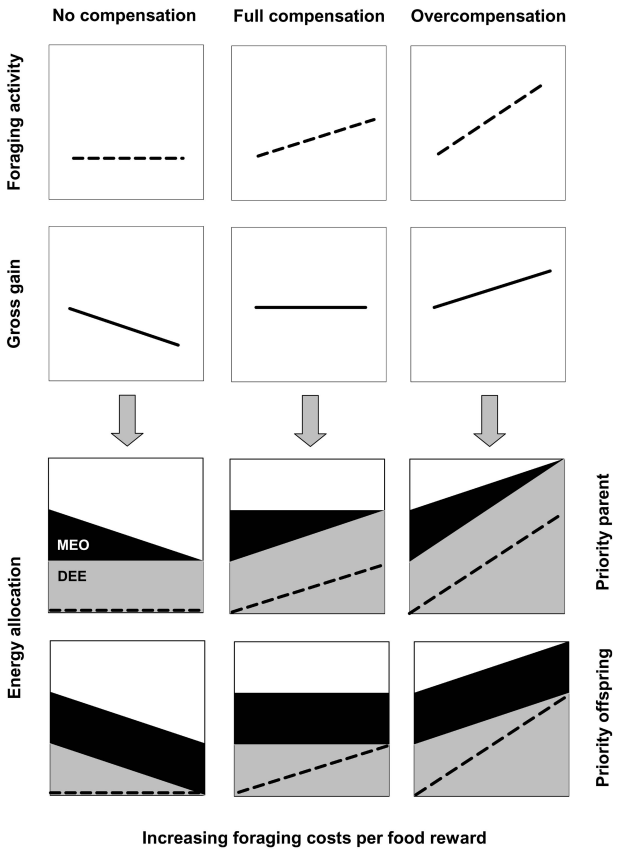

Figure 1: Schematic models of energy allocation for breeding mammals faced with varying foraging costs per reward. The top panels show responses in foraging activity (row 1) and resultant gross gain (row 2) under increasing foraging costs. The bottom panels show energy allocation strategies possible if mothers preferentially invest in their own somatic maintenance (row 3) or their offspring (row 4). In the bottom panels, changes in total energy intake (upper boundary) and daily energy expenditure (DEE, *gray shading*) are associated with different patterns of energy allocation to reproduction, that is, milk energy output (MEO, *black shading*). Energy required for activity is shown by a dashed line. The models are ordered from left to right to indicate the degree of behavioral and metabolic compensation shown by the mother (no compensation, full compensation, overcompensation).

the proportional investment in self versus offspring and verified that integrated estimates of milk production explained variation in litter growth. Our approach explores the metabolic underpinnings of reproductive consequences of environmental quality, which are of fundamental importance to the evolution of life-history tradeoffs.

Material and Methods

Animals and Housing

Female mice (*Mus musculus domesticus*, outbred Hsd: ICR[CD-1] strain) were kept as described by Schubert et

al. (2008). Briefly, they were individually housed in Plexiglas cages (20 cm × 20 cm × 30 cm) with plastic running wheels (14 cm in diameter; code 0131 Savic, Kortrijk, Belgium), at 21° ± 1°C (artificial 12L : 12D cycle, lights on 1100 hours GMT + 1) and weighed daily ~1 h before lights out. Food pellets (TestDiet 5TUM/PJAI, Sandown Chemicals, Hampton, UK) were either given ad lib. or dispensed at a fixed reward ratio in response to wheel running (Series 3 Programmable Controller, General Electric; Med Associates Pellet Dispenser ENV-203, Sandown Scientific, Hampton, UK). In a sense, all mice were fed ad lib. regardless of treatment, because they were free to eat or work for food whenever they chose. Wheel-running activity (measured in revolutions [rev]) was logged in 2-min activity bins with a computerized event-recording system (ERS). All animal care and treatment procedures were in accordance with Dutch regulations (University of Groningen DEC license 4484A).

Experimental Design

We selected female mice that had successfully bred once in the experimental cages. Ad lib., nonbreeding food intake and spontaneous wheel-running activity were measured for 1 wk. With these data, we calculated each animal's "baseline reward rate" (rev pellet^{-1}) from spontaneous wheel-running activity (mean ± SE = 168 ± 9 rev pellet^{-1}) divided by ad lib. food intake. To prevent pup killing due to stress, we trained animals to run for pellets before the experiment; they were given a 10% daily increase in foraging costs per reward until they reached 150% of baseline, followed by a recovery week with ad lib. food. Two weeks after pairing with a male (i.e., during the final week of gestation), females were put on an experimental regime if they had gained at least 5 g in body mass. Mice were randomly assigned to four groups with different foraging requirements (initial $n = 7$ per group) based on their individual baseline reward rates: A, fed ad lib., with access to a running wheel; L, foraging at a reward rate one-third of baseline; M, foraging at two-thirds of baseline; H, foraging at baseline. They stayed on these regimes until litters were weaned, 19–22 days postpartum. For ethical reasons, if a female or litter lost more than 2 g in a day, it was given extra food (~3 g). Some females did not produce viable litters or there were technical problems interfering with data collection; in five of seven instances, these litters died before day 2. In the data analysis, we used only females with litters surviving the first week of life (analyzed sample sizes per group: A = 6, L = 6, M = 4, H = 5). The ranges of foraging costs (rev pellet^{-1}) for these individuals were 0 for A, 49–85 for L, 97–138 for M, and 105–212 for H.

Metabolic Measurements

We measured the daily energy expenditure (DEE, kJ day^{-1}) of females at peak lactation (day 17 ± 1) with the doubly labeled water (DLW) method (Lifson and McClintock 1966; Speakman 1997), as described by Schubert et al. (2008). Briefly, animals were weighed to the nearest 0.1 g and injected intraperitoneally with ~0.1 g of enriched water (37.6% ^{2}H and 60.6% ^{18}O). Duplicate 15-μL samples of tail-tip blood were collected in flame-sealed glass capillary tubes after a 1-h equilibration period ("initial") and 24 h later ("final," in triplicate). $^{2}H/^{1}H$ and $^{15}O/^{16}O$ ratios in blood samples were measured by mass spectrometry at the University of Groningen's Center for Isotope Research. The rate of CO_2 production for each animal was calculated from Speakman's (1997) single-pool model equation (7.17). CO_2 production was converted to energy expenditure assuming an energetic equivalent of 22.0 kJ L^{-1} CO_2.

We measured the resting metabolic rate of the females when the pups were 16 ± 1 days old, using the open-flow respirometry system described by Oklejewicz et al. (1997) and Schubert et al. (2008). Our protocol was modified to measure animals at 30° ± 1°C (thermoneutrality) for 3–4 h, ending at least 1 h before the start of the active circadian phase; females did not have access to food or water, and their litters remained in the home cage. These are the same conditions typically used to characterize basal metabolic rate and are the accepted standard for lactating mice (Johnson et al. 2001*b*), because they allow for short measurements on the mother without harming the offspring. Metabolic rate (MR, kJ h^{-1}) was calculated from O_2 consumption and CO_2 production by using the formula of Romijn and Lokhorst (1961). The MR decreased over time and usually reached a stable minimum after 3 h. We defined the resting metabolic rate at peak lactation (RMR, kJ h^{-1}) as the lowest value of a 30-min running mean of MR. Metabolizable energy intake (MEI) was the gross energy intake (18.16 kJ g^{-1}) multiplied by a standard assimilation efficiency (80.8%; Johnson et al. 2001*a*). Milk energy output (MEO) was estimated from the difference between MEI and DEE (Król and Speakman 2003). This reference method of MEO determination, the results of which are strongly correlated with the litter energy budget under ad lib. conditions, is the best empirically validated technique available (Król and Speakman 2003).

Data Handling and Statistical Analyses

We analyzed data using Statistica 6.1 (StatSoft, Tulsa, OK), after checking for normality and arcsine-transforming proportional measures to $\sin^{-1}(y^{1/2})$. Two-tailed P values of ≤ 0.05 were considered statistically significant. We compared treatment groups by using ordered heterogeneity

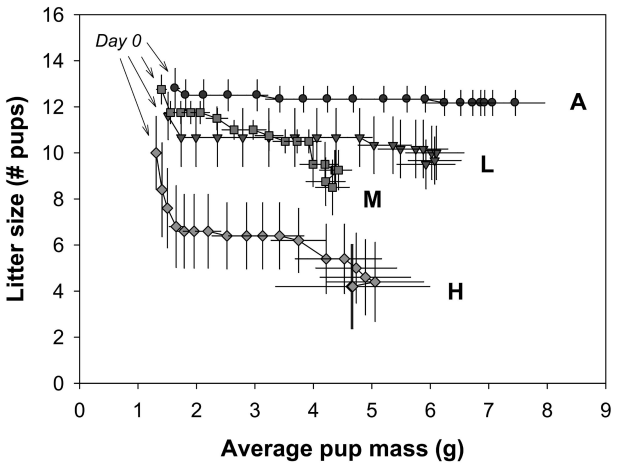

Figure 2: Growth trajectories from birth to day 18 postpartum for litters of female mice experiencing varying foraging costs per reward. Symbols indicate maternal foraging costs: none (ad lib. feeding; *A*, *circles*), low (*L*, *triangles*), medium (*M*, *squares*), or high (*H*, *diamonds*). Data points are daily measurements beginning at day 0 (top left corner). Bars show SE for litter size and average pup mass.

tests (OHTs; Rice and Gaines 1994). This test extends heterogeneity tests such as ANOVA to increase statistical power when treatment categories are ordered, information that is not used in an ANOVA. It combines the *P* value from the heterogeneity test with a correlation calculated between the treatment ranks and the ranks of the response variable means for the different treatments. Formally, significance is evaluated by using the composite test statistic $r_s P_c$, where r_s is the Spearman correlation between group means and the treatment ranks and P_c is the complement of the *P* value from the ANOVA ($1 - P_{HT}$). We used the graphs provided by Rice and Gaines (1994) to determine the two-tailed *P* values associated with the $r_s P_c$ statistic for four ordered treatment groups. Additional covariate analyses were performed with general linear models (GLMs), beginning with linear predictors and checking for significant quadratic effects. To test whether our linear models dealt completely with variation between foraging-cost groups, we then tested whether the addition of a group factor significantly improved the fit of our models.

All means are shown with their standard errors. Data were analyzed at day 0 (parturition), peak lactation (mean days 13–16), or over the entire breeding period (days 0–18). Variables representing the entire breeding event were integrated by taking the sum of daily changes (e.g., cumulative growth of all pups in a litter) or the difference between day 0 and day 18 (e.g., change in litter mass). In addition to measuring MEO directly at peak lactation (described above), we modeled MEO for the entire reproductive period. In doing so, we assumed DEE to be predicted by body mass throughout lactation; all energy used for growth (body remodeling) and milk biosynthesis is subsumed within the DEE, which limits our assumptions to this single parameter. We used daily mass records and the predictions from equations relating metabolism to mass at peak lactation to back-estimate daily metabolic parameters (RMR, DEE) over the entire breeding period;

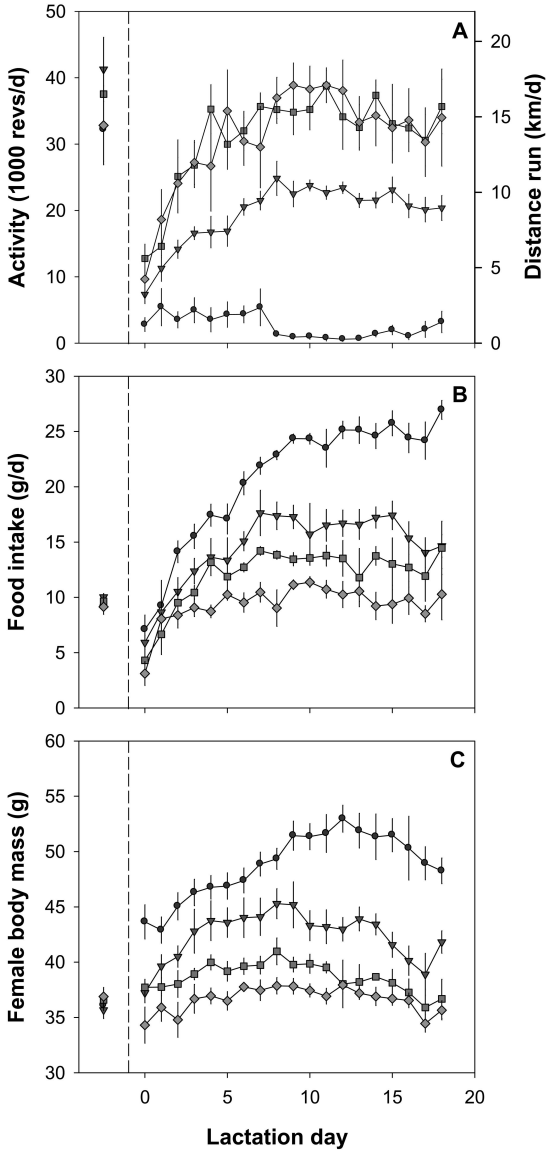

Figure 3: Development of activity (*A*), food intake (*B*), and body mass (*C*) of female mice faced with varying foraging costs per reward during reproduction. Symbols indicate groups experiencing ad lib. feeding (*circles*) or low (*triangles*), medium (*squares*), or high (*diamonds*) foraging costs. Data in the left-hand panels show mean nonbreeding values in the week before pairing. Bars indicate SE.

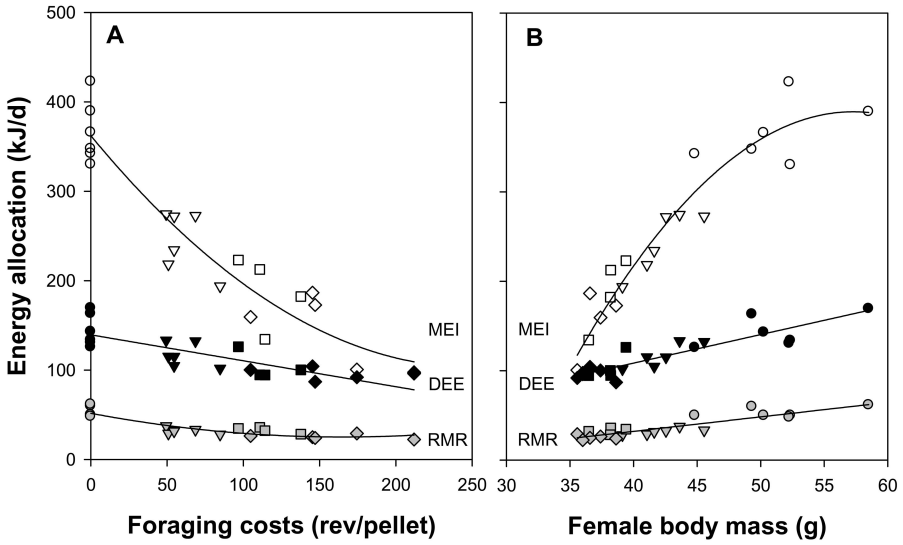

Figure 4: Energy balance at peak lactation of female mice experiencing ad lib. feeding (*circles*) or low (*triangles*), medium (*squares*), or high (*diamonds*) foraging costs per reward. MEI, DEE, and RMR represent metabolizable energy intake, daily energy expenditure, and resting metabolic rate, respectively. *A*, Relationship between energy allocation and activity. *B*, Relationship between energy metabolism and maternal body mass. DEE and RMR are based on measurements using doubly labeled water and respirometry (see "Metabolic Measurements"); other data are parameter means averaged over days 13–16.

total MEO was similarly estimated by subtracting estimated daily DEE from daily MEI.

Results

Reproductive Output

The experimental increase of foraging costs (per reward) drastically decreased reproductive output (fig. 2). At birth, there was already a negative association between foraging costs and litter mass (OHT: $F_{3,15} = 3.58$, $r_s P_c = 0.77$, $P < .05$), due largely to lower average pup mass (OHT: $F_{3,15} = 5.14$, $r_s P_c = 0.99$, $P < .0001$), without a significant contribution of birth litter size (OHT: $F_{3,15} = 1.37$, $r_s P_c = 0.57$, $P > .1$). Subsequent pup mortality increased with rising foraging costs, which led to a strong reduction in litter size by day 18 (OHT: $F_{3,17} = 7.96$, $r_s P_c > 0.99$, $P < .0001$). Pup and litter mass at day 18 also decreased with rising foraging costs (OHT, pup mass: $F_{3,17} = 3.53$, $r_s P_c = 0.77$, $P < .05$; OHT, litter mass: $F_{3,17} = 29.06$, $r_s P_c > 0.99$, $P < .0001$).

Maternal Responses: Foraging Effort, Body Mass, and Energy Expenditure

To understand the reproductive consequences of environmental quality in energetic terms, we first quantified the effect on foraging effort and food intake. Mice fed ad lib. (group A) showed almost no wheel-running activity at peak lactation (days 13–16), while the other groups dramatically elevated their activity (fig. 3*A*; OHT: $F_{3,17} = 67.60$, $r_s P_c = 0.80$, $P < .02$). The three groups that had to forage for food spent, on average, more time running than ad lib. females (calculated from the proportion of ERS bins showing activity: A = 2.6 ± 0.6, L = 11.7 ± 0.5, M = 12.4 ± 0.8, H = 12.0 ± 0.4 h day^{-1}; OHT: $F_{3,17} = 63.86$, $r_s P_c = -0.80$, $P < .02$). Food intake declined with increasing foraging costs (fig. 3*B*; OHT: $F_{3,17} = 37.03$, $r_s P_c > 0.99$, $P < .0001$), as did body mass gain (fig. 3*C*; OHT: $F_{3,17} = 29.20$, $r_s P_c > 0.99$, $P < .0001$). High foraging costs reduced both total maternal energy intake and expenditure (fig. 4). At peak lactation, MEI, MEO, DEE, and RMR all scaled negatively with foraging costs and positively with body mass; the relationships between the various energy turnover measures and mass were statistically explained by linear or quadratic effects of predictor variables (table 1). Adding group as a factor did not increase the variance explained by these models significantly (table 2), except in the case of both models for RMR.

Energy Allocation to Self and Offspring

The proportion of DEE allocated to RMR decreased with rising foraging costs (A = 0.37 ± 0.01, L = 0.27 ± 0.01, M = 0.32 ± 0.02, H = 0.27 ± 0.01; OHT: $F_{3,17} = 14.45$, $r_s P_c = -0.80$, $P < .02$), while at the same time, the pro-

Table 1: Maternal metabolic traits in relation to foraging costs and whole-body mass at peak lactation

GLM	Intercept ± SE	Slope (x) ± SE	Slope (x^2) ± SE	R^2	Model F	df	P
Versus foraging costs:							
MEI	362.29 ± 12.74	−2.07 ± .33	.004 ± .002	.89	74.71	2, 18	<.0001
MEO	208.43 ± 11.02	−1.06 ± .11	NS	.83	91.04	1, 19	<.0001
DEE	139.40 ± 4.84	−.29 ± .05	NS	.65	35.15	1, 19	<.0001
RMR	51.66 ± 2.03	−.32 ± .05	.001 ± .0003	.82	42.19	2, 18	<.0001
Versus body mass:							
MEI	−1,503.93 ± 303.62	66.09 ± 13.54	−.58 ± .15	.93	111.38	2, 18	<.0001
MEO	−1,392.03 ± 323.10	58.73 ± 14.41	−.53 ± .16	.87	59.16	2, 18	<.0001
DEE	−19.07 ± 16.30	3.19 ± .38	NS	.79	71.43	1, 19	<.0001
RMR	−32.58 ± 7.99	1.62 ± .19	NS	.80	76.51	1, 19	<.0001

Note: Results of linear or quadratic models for foraging costs (revolutions pellet^{-1}) and body mass (g) are presented. Nonsignificant terms excluded from the final model are denoted NS. Analyses were performed on a data set of 21 observations. GLM = general linearized model; MEI = metabolizable energy intake (kJ day^{-1}); MEO = milk energy output (kJ day^{-1}); DEE = daily energy expenditure (kJ day^{-1}); RMR = resting metabolic rate (kJ day^{-1}).

portion of MEI allocated to DEE increased (A = 0.40 ± 0.02, L = 0.48 ± 0.02, M = 0.57 ± 0.05, H = 0.72 ± 0.10; OHT: $F_{3,17} = 7.00$, $r_s P_c > 0.99$, $P < .0001$). Mass-specific RMR (kJ day^{-1} g^{-1}) at peak lactation declined with rising foraging costs (OHT: $F_{3,17} = 18.35$, $r_s P_c = -0.80$, $P < .02$); however, there was no effect on mass-specific DEE (kJ day^{-1} g^{-1}; OHT: $F_{3,17} = 0.64$, $r_s P_c = -0.40$, $P > .1$). Females with high foraging costs had average peak RMR and DEE of only 50% and 66% that of ad lib. animals, respectively.

At peak lactation, milk energy output was strongly correlated with litter size, litter mass, and average pup mass (fig. 5). Cumulative energy allocation to offspring over days 0–18 declined with rising foraging costs (fig. 6, *bottom*; OHT: $F_{3,17} = 26.72$, $r_s P_c > 0.99$, $P < .0001$), to a greater extent than integrated measures of DEE and RMR (fig. 6, *bottom*; OHT, RMR: $F_{3,17} = 48.00$, $r_s P_c > 0.99$, $P < .0001$; OHT, DEE: $F_{3,17} = 12.29$, $r_s P_c > 0.99$, $P < .0001$). Litter growth closely paralleled patterns of energy allocation (fig. 6, *top*).

Overall, there was a strong link between foraging costs, energy allocation to reproduction, and offspring growth. MEO relative to MEI declined with increasing foraging costs, both at peak lactation (A = 0.60 ± 0.04, L = 0.52 ± 0.03, M = 0.43 ± 0.10, H = 0.28 ± 0.19; OHT: $F_{3,17} = 5.98$, $r_s P_c > 0.99$, $P < .0001$) and over the entire reproductive period (A = 0.57 ± 0.02, L = 0.47 ± 0.04, M = 0.44 ± 0.04, H = 0.32 ± 0.07; OHT: $F_{3,17} = 18.70$, $r_s P_c > 0.99$, $P < .0001$). Total litter growth scaled linearly with total MEO (fig. 7). We defined litter growth efficiency as the energetic content of growth per milk energy input, assuming a value of 8.95 kJ g^{-1} for pup biomass (Johnson et al. 2001*a*). Growth efficiency (pup biomass energy yield over milk energy) integrated over days 0–18 was inde-

Table 2: General linear models fitting metabolic traits of female mice at peak lactation against foraging costs and whole-body mass

	Simple model			Model including group			Comparison		
GLM	R^2	SSE	df	R^2	SSE	df	F	P	Final model
Versus foraging costs:									
MEI	.89	18,948.72	18	.90	17,504.85	15	.41	.75	Quadratic
MEO	.83	19,511.42	19	.86	15,548.09	16	1.36	.29	Linear
DEE	.65	3,764.46	19	.70	3,248.90	16	.85	.49	Linear
RMR	.82	479.22	18	.90	260.57	15	4.20	.02	Quadratic + group
Versus body mass:									
MEI	.93	13,177.06	18	.94	10,529.01	15	1.26	.32	Quadratic
MEO	.87	14,921.83	18	.89	12,440.68	15	1.00	.42	Quadratic
DEE	.79	2,254.14	19	.80	2,193.73	16	.15	.93	Linear
RMR	.80	542.21	19	.92	225.30	16	7.50	.00	Linear + group

Note: Simple models included foraging costs (rev pellet^{-1}) or body mass (g)—fitted as linear or quadratic effects—as covariates. Group was then added a factor, to test whether this increased the total explained variance of the model. All analyses were performed on a data set of 21 observations. GLM = general linear model; SSE = error sum of squares; MEI = metabolizable energy intake (kJ day^{-1}); MEO = milk energy output (kJ day^{-1}); DEE = daily energy expenditure (kJ day^{-1}); RMR = resting metabolic rate (kJ day^{-1}).

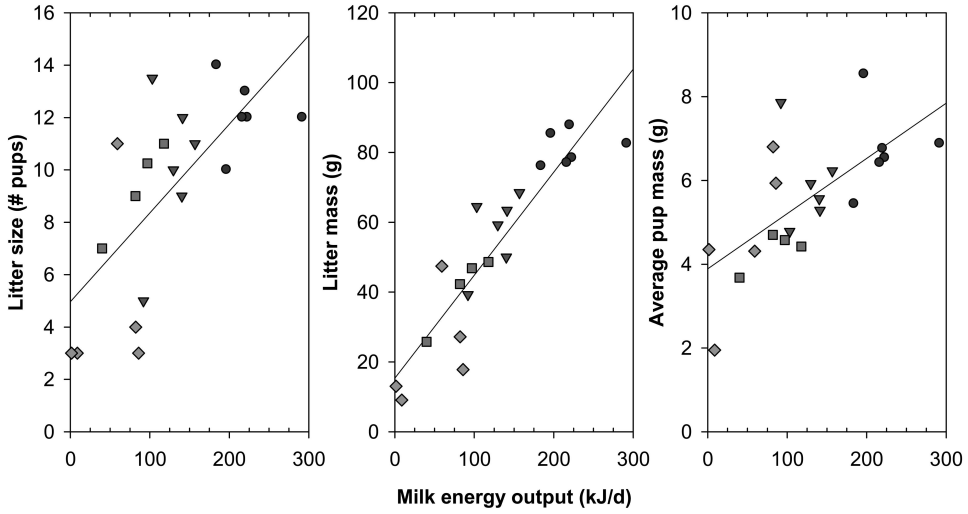

Figure 5: Relationship between milk energy output and litter characteristics at peak lactation. Litter data are means averaged over days 13–16 postpartum. Symbols represent litters for mothers facing ad lib. conditions (*circles*) or low (*triangles*), medium (*squares*), or high (*diamonds*) foraging costs per reward. Milk energy output significantly predicted litter size ($y = 0.034x + 4.96$, $R^2 = 0.49$, $F_{1,19} = 18.40$, $P = .0004$), litter mass ($y = 0.30x + 15.35$, $R^2 = 0.82$, $F_{1,19} = 87.46$, $P < .0001$), and average pup mass ($y = 0.01x + 3.89$, $R^2 = 0.44$, $F_{1,19} = 14.99$, $P = .001$).

pendent of foraging costs (overall = 0.21; A = 0.20 ± 0.02, L = 0.22 ± 0.04, M = 0.20 ± 0.01, H = 0.21 ± 0.06; OHT: $F_{3,17} = 0.39$, $r_s P_c = -0.09$, $P \gg .1$). This is in agreement with the finding that the regression line in figure 7 not only is linear but also has an intercept that does not differ significantly from 0 (i.e., the line can be assumed to go through the origin).

Discussion

Breeding animals confronted with an increase in foraging costs face two decisions: (1) how hard to work for their food and (2) how to divide the energy they earn between self and offspring. We found that lactating female mice increased foraging effort in response to declining environmental quality (fig. 3*A*) but that this increase in foraging effort was not sufficient even to maintain food intake (fig. 3*B*), let alone to recoup the extra energy spent on foraging. With regard to the allocation models in figure 1, we therefore conclude that females followed a strategy of partial compensation for poor environmental quality. This result is in broad agreement with earlier findings in nonreproducing birds and mammals (see Wiersma and Verhulst 2005 for an overview).

Trade-offs between investment in self and offspring are a basic precept of life-history theory (Williams 1966; Roff 1992), but they are not easily quantified, and to the best of our knowledge, this is the first study to dissect maternal energy allocation in response to foraging-cost variation in a breeding animal. Deteriorating environmental quality led to a reduction in energy intake, the largest share of which came at the expense of energy allocated to milk production (fig. 6). This is in qualitative agreement with what one would expect, since the total energy budget has to be divided between self and offspring, and energy available for reproduction is the surplus after maintenance and activity have been provided for. The reduced milk production in low-quality environments fully explained the environmental effect on reproductive output, in the sense that there was no evidence of additional environmental effects (fig. 7). Thus, our data explain the link between environmental quality and reproductive output via maternal foraging effort and resource allocation.

Pups rely entirely on maternal milk as their energy source, and total litter energy budget correlates well with MEO (measured with respirometry at 30°C; Król and Speakman 2003). We found that the efficiency of milk energy conversion to offspring mass was independent of environmental quality (fig. 7), suggesting that proportional energy use for growth by the offspring was independent of the amount of energy received. This is interesting, given that pup growth, like maternal milk production, relies on surplus energy available after maintenance and activity are provided for. Consequently, when the amount of energy pups receive is low, one would expect them to spend a smaller proportion of the total energy available on growth. Moreover, it seems intuitively likely that factors such as the number and mass of the pups, which varied with environmental quality (fig. 2), would influence energy requirements for maintenance and activity and thereby affect

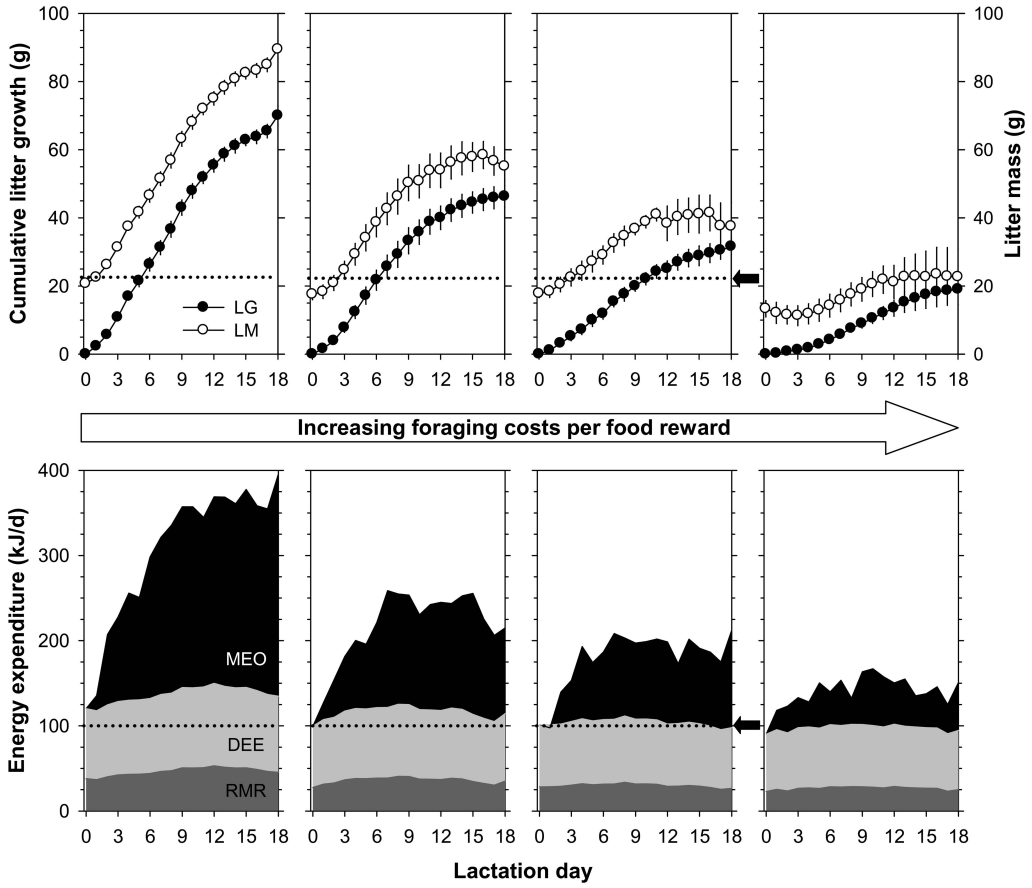

Figure 6: Reproductive output and maternal energy allocation. *Top*, rising foraging costs per reward decreased the rate of litter growth (LG, *filled circles*) over time. Litter mass (LM, *open circles*) increased more slowly under elevated foraging costs. *Bottom*, increased foraging costs altered maternal energy allocation. Maintenance costs represented by daily energy expenditure (DEE) and resting metabolic rate (RMR) decreased; milk energy output (MEO) was drastically reduced. Energetic estimates are derived from daily measurements of maternal food intake and body mass. On the critical assumption that mass-energy scaling remained constant throughout reproduction, we used regression equations for DEE and RMR versus body mass at peak lactation (see table 1 for equations) to predict energy requirements of each animal on a daily basis. The upper boundary of the shaded area represents metabolizable energy intake (MEI), the dark gray shaded area represents RMR, and the sum of the two gray areas represents DEE. MEO is the difference between MEI and DEE, equivalent to the surplus energy available for transfer to offspring as milk.

milk energy conversion efficiency. Our estimates of growth efficiency suggests that, regardless of maternal foraging treatment, pups convert ~21% of the energy they obtain to body growth and spend the rest on their own metabolic maintenance, thermoregulation, and activity. Regardless of the mechanism, the finding that the efficiency of milk energy conversion to offspring mass was invariant with respect to environmental quality makes our results more general. In our study, foraging conditions changed late in pregnancy, and hence females had little opportunity to adjust litter size to foraging conditions. The invariant milk energy conversion efficiency suggests that our conclusions regarding litter mass production are, in fact, largely independent of whether the opportunity for litter size adjustments was available. On a practical level, the invariant milk energy conversion efficiency implies that the milk energy output can be inferred directly from litter growth. This may greatly facilitate the future study of mammalian reproductive effort, although further work is obviously needed to establish how general this finding is.

It remains unclear why animals faced with high foraging costs did not compensate fully by increasing their activity and elevating total energy expenditure to "pay" for this activity (Wiersma et al. 2005). Breeding females were clearly motivated to run when necessary, since they spent much more time running when environmental quality deteriorated. It is possible, however, that animals encountered constraints limiting their running activity, since females in the high-foraging-cost group (H) did not run more than those in the intermediate-cost group (M).

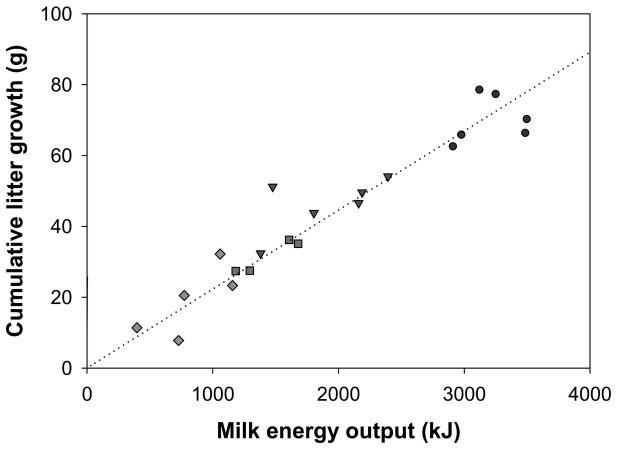

Figure 7: Estimate of the relationship between total milk energy output (MEO) and total litter growth from day 0 to day 18. MEO is the sum of daily MEO values. Symbols represent individual litters of mothers faced with ad lib. conditions (*circles*) or low (*triangles*), medium (*squares*), or high (*diamonds*) foraging costs per reward. The regression line ($y = 0.022x$) was drawn through the origin because the intercept did not deviate significantly from 0 (general linear model: intercept = 3.76 ± 3.18, df = 20, $t = 1.18$, $P = .25$; slope = 0.021 ± 0.001, df = 20, $t = 13.99$, $P < .0001$, $R^2 = 0.91$).

While mice fed ad lib. spent, on average, only 10% of their time in the running wheel, females in the other groups spent ~50% of their time in the wheel. Sleeping, suckling/warming young, and grooming are among the many activities likely to limit the time available for wheel running each day. Such constraints may explain why animals faced with high foraging costs did not increase their foraging effort further. In addition to constraints, optimality considerations could explain why females did not increase foraging effort even more. For example, why did mice in the L group run less than those in the M and H groups (fig. 3), despite the fact that their litters developed less well when compared to litters of animals that did not have to work for food (fig. 2)? Not all reproductive effort should be worth the cost to parents, because altered allocation during breeding could reduce investment in necessary maintenance processes (i.e., protection from oxidative stress: Wiersma et al. 2004; somatic repair: Nilsson and Svensson 1996; Wiersma and Verhulst 2005; immune function: Sheldon and Verhulst 1996; Deerenberg et al. 1997) and hence future reproductive output. Thus, in a life-history context, we can interpret our results as evidence that breeding animals restrained themselves to avoid paying a future fitness cost (Drent and Daan 1980).

Mammalian lactation relies on high energy throughput, and under ad lib. circumstances, female mice invest heavily in tissues necessary for metabolizing food and producing milk, as illustrated by their increase in mass (i.e., figs. 3*C*, 4*B*). When faced with high foraging costs, experimental animals compensated by investing less in the body mass increases necessary for milk production. This can be partially viewed as an energy-saving strategy, since a smaller body requires a lower resting metabolism. However, such savings are also necessarily associated with decreases in milk production. When maternal body mass was corrected for, the effect of foraging costs on DEE all but disappeared, while the declining effect on MEO was still present. This further confirms that the offspring have to bear the burden of the reduction in maternal energy budget in poorer environments. Our allocation results show that maternal effort—classically defined as the proportion of total energy allocated to reproduction (e.g., Hirschfield and Tinkle 1975)—is reduced when environmental quality is poor. Thus, in natural populations, we would predict that an increase in foraging costs would result in a disproportionate decrease in reproductive output.

Acknowledgments

This study was initiated in collaboration with the late G. Henk Visser. We thank E. Rutgers and R. Gordijn for help with data collection. G. Overkamp created and maintained the foraging setup and the respirometry system. J. Speakman provided invaluable guidance in analysis of DLW data. G.V. thanks P. Barf and M. Oostindjer for additional input. S.V. was supported by an NWO (Netherlands Organization for Scientific Research) Vici grant, K.A.S. and L.M.V. by the University of Groningen, and S.D. by European Community grant LSHM-CT-2006-018741 (EUCLOCK).

Literature Cited

Boggs, C. L. 1992. Resource allocation: exploring connections between foraging and life history. Functional Ecology 6:508–518.

Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68:203–220.

Daan, S., D. Masman, A. Strijkstra, and G. J. Kenagy. 1991. Daily energy turnover during reproduction in birds and mammals: its relationship to basal metabolic rate. Pages 1976–1987 *in* Acta XX Congressus Internationalis Ornithologici. Ornithological Congress Trust Board, Wellington, New Zealand.

Deerenberg, C., V. Arpanius, S. Daan, and N. Bos. 1997. Reproductive effort decreases antibody responsiveness. Proceedings of the Royal Society B: Biological Sciences 264:1021–1029.

de Jong, G., and A. J. van Noordwijk. 1992. Acquisition and allocation of resources: genetic (co)variances, selection, and life histories. American Naturalist 139:749–770.

Drent, R., and S. Daan. 1980. The prudent parent. Ardea 68:225–252.

Gittleman, J. L., and S. D. Thompson. 1988. Energy allocation in mammalian reproduction. American Zoologist 28:863–875.

Hirschfield, M. F., and D. W. Tinkle. 1975. Natural selection and the

evolution of reproductive effort. Proceedings of the National Academy of Sciences of the USA 72:2227–2231.

Johnson, M. S., S. C. Thomson, and J. R. Speakman. 2001*a*. Limits to sustained energy intake. I. Lactation in the laboratory mouse *Mus musculus*. Journal of Experimental Biology 204:1925–1935.

———. 2001*b*. Limits to sustained energy intake. II. Inter-relationships between resting metabolic rate, life-history traits and morphology in *Mus musculus*. Journal of Experimental Biology 204: 1937–1946.

Król, E., and J. R. Speakman. 2003. Limits to sustained energy intake VII. Milk energy output in laboratory mice at thermoneutrality. Journal of Experimental Biology 206:4267–4281.

Lemon, W. C. 1991. Fitness consequences of foraging behaviour in the zebra finch. Nature 352:153–155.

Lifson, N., and R. McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. Journal of Theoretical Biology 12:46–74.

Markman, S., B. Pinshow, and J. Wright. 2002. The manipulation of food resources reveals sex-specific trade-offs between parental self-feeding and offspring care. Proceedings of the Royal Society B: Biological Sciences 269:1931–1938.

Martin, T. E. 1987. Food as a limit for breeding birds: a life-history perspective. Annual Review of Ecology and Systematics 18:453–487.

Nilsson, J.-Å., and E. Svensson. 1996. The cost of reproduction: a new link between current reproductive effort and future reproductive success. Proceedings of the Royal Society B: Biological Sciences 263:711–714.

Oklejewicz, M., R. A. Hut, S. Daan, A. S. Loudon, and A. J. Stirland. 1997. Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamsters. Journal of Biological Rhythms 12: 413–422.

Perrigo, G. 1987. Breeding and feeding strategies in deer mice and house mice when females are challenged to work for their food. Animal Behaviour 35:1298–1316.

Rice, W. R., and S. D. Gaines. 1994. Extending nondirectional heterogeneity tests to evaluate simply ordered alternative hypotheses. Proceedings of the National Academy of Sciences of the USA 91: 225–226.

Ricklefs, R. E., and M. Wikelski. 2002. The physiology/life-history nexus. Trends in Ecology & Evolution 17:462–468.

Roff, D. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York.

Romijn, C., and W. Lokhorst. 1961. Some aspects of energy metabolism in birds. Pages 49–58 *in* E. Brouwer and A. J. H. van Es, eds. Proceedings Second Symposium on Energy Metabolism. European Association for Animal Production, Wageningen.

Schubert, K. A., L. M. Vaanholt, F. Stavasius, G. E. Demas, S. Daan, and G. H. Visser. 2008. Female mice respond differently to costly foraging vs. food restriction. Journal of Experimental Biology 211: 2214–2223.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunity: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution 11:317–321.

Sikes, R. S. 1995. Costs of lactation and optimal litter size in northern grasshopper mice (*Onychomys leucogaster*). Journal of Mammalogy 76:348–357.

Speakman, J. R. 1997. Doubly labelled water: theory and practice. Chapman & Hall, London.

———. 2008. The physiological costs of reproduction in small mammals. Philosophical Transactions of the Royal Society B: Biological Sciences 363:375–398.

Speakman, J. R., and E. Król. 2005. Limits to sustained energy intake. IX. A review of hypotheses. Journal of Comparative Physiology B 175:375–394.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford.

Vaanholt, L. M., B. De Jong, T. Garland Jr., S. Daan, and G. H. Visser. 2007. Behavioural and physiological responses to increased foraging effort in male mice. Journal of Experimental Biology 210: 2013–2024.

Wiersma, P., and S. Verhulst. 2005. Effects of intake rate on energy expenditure, somatic repair and reproduction of zebra finches. Journal of Experimental Biology 208:4091–4098.

Wiersma, P., C. Selman, J. R. Speakman, and S. Verhulst. 2004. Birds sacrifice oxidative protection for reproduction. Proceedings of the Royal Society B: Biological Sciences 271:S360–S363.

Wiersma, P., H. M. Salomons, and S. Verhulst. 2005. Metabolic adjustments to increasing foraging costs of starlings in a closed economy. Journal of Experimental Biology 208:4099–4108.

Williams, G. C. 1966. Adaptation and natural selection. Princeton University Press, Princeton, NJ.

Williams, T. D., and F. Vézina. 2001. Reproductive energy expenditure, intraspecific variation and fitness in birds. Pages 355–406 *in* V. Nolan Jr., ed. Current ornithology. Kluwer Academic/Plenum, New York.

Associate Editor: Allen J. Moore
Editor: Michael C. Whitlock